persistence in MDA-MB-231 and BT-549 cell lines. Both cell lines have the same metastatic profile (39% frequency of bone metastasis and 25% frequency of brain metastasis), and show similar results on EMM mimicking bone and brain tissue. As expected from in vivo clinical data, MDA-MB-231 cells are larger, less spherical, adhere faster, migrate faster, and persist in one direction longer on bone mimics than on brain mimics. BT-549 cells show similar patterns, but differ in cell area and persistence. However, these differences are not significant, and the former may be due to a specific blebbing form of migration observed. We are currently engineering a lung EMM, and examining the role of tissue stiffness via PEG-PC hydrogels in dictating tissue tropism in metastasis.

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Microfluidic Device for Controlled Fluid Switching to be used with Chemically Powered Molecular Motors on Surface Bound Tracks

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For many studies it is desirable to controllably and in real time change the chemical environment surrounding a biological sample, while continuously microscopically observing reactions that occur. Here we have developed such a technique to satisfy a particular set of restrictions related to real-time studies of processive molecular motor motion on surface-immobilized tracks. Specifically, this synthetic protein motor design requires switching of three or more solutions in any desired order, in order to be able to control the direction of motor motion. We have designed, theoretically modeled and experimentally tested this microfluidic device, which utilizes an on-chip switching method to avoid issues of solution contamination due to backflow commonly associated with the use of valves. The dynamics of the fluid near the surface of the channel has been studied theoretically using finite element modeling. The switching of fluid containing fluorescent molecules has been imaged in bulk with epifluorescent microscopy as well as at the surface of the channel using TIRF microscopy. The lower limit of the fluid speed that permits the fluid switching near the surface has been tested as well as the upper limit of the frequency of switching, which is diffusion limited. Besides our desired application to artificial molecular motors, the type of device developed here can be used for any experiment that requires controlled changes in the chemical environment of surface-adhered samples.

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Inexpensive Raft Arrays for Cell Patterning and Cloning

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An easy to use, inexpensive, efficient system has been developed for the combined purpose of patterned cell culture and cell separations. The system consists of a specialized flexible array of micron-scale array elements created by a unique soft lithography process compatible with a host of biocompatible polymers, including polystyrene. Low autofluorescence, standardized tissue culture surfaces can be created and tailored to the cell type of interest. The concave array elements, termed rafts, are fabricated within poly(dimethylsiloxane) (PDMS) microwells and are individually releasable using a needle inserted through the PDMS substrate with a micromanipulator. The array enables efficient cell analysis and isolation even with small populations containing as few as 1,000 cells. Cells plated on the arrays are localized to individual rafts throughout the culture period by virtue of the PDMS microwells. While the cells are prevented from migrating and intermixing, they share a common fluidic microenvironment in contrast to standard multiwell plates. Standard microscopic imaging is used to identify and track cells or colonies over time to allow selection based on temporal or other characteristics. Those of interest are released by inserting the needle through the compliant PDMS substrate to dislodge the raft on which the cell(s) of interest lies. The hardened polymer material forming the raft protects the cells growing on its upper surface from harm by the needle. Selective release of the individual elements along with the resident cells has been enhanced with the use of polymers doped with ferromagnetic nanoparticles to achieve high collection efficiencies of viable cells under the influence of a magnetic field. In the current state of development, collection efficiency and single-cell cloning rates as high as 95% have been achieved. Among other attributes, the array enables rapid cloning to produce cell lines with desired characteristics.

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Formation and Characterization of Ultrathin Films Containing Amyloidogenic Proteins

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Ultrathin films are useful for coating materials and controlling drug delivery processes. Here, we explore the use of two-dimensional amyloidal networks as biodegradable ultrathin films. In a first step, we have studied the lateral aggregation and fibril formation of insulin that is adsorbed at and confined within planar polyelectrolyte multilayers containing poly(diallyldimethylammonium chloride) (PDDA), poly(styrenesulfonic acid) (PSS), and hyaluronic acid (Hyal). Si-PDDA-PSS-(Ins-PSS)$_x$ and Si-PDDA-PSS-(Ins-Hyal)$_x$ multilayers have been prepared and characterized in the hydrated state by using X-ray reflectometry, ATR-FTIR spectroscopy and confocal fluorescence microscopy. The obtained data demonstrate a successful build-up of insulin-polyelectrolyte multilayers on silicon wafers that grow strongly in thickness upon insulin adsorption on PSS and Hyal layers. The secondary structure analysis of insulin, based on the insulin infrared amide I' band, indicates intermolecular β-sheet formation within the multilayers at 70 °C and pH = 2, i.e. at conditions that promote amyloid fibrils rich in β-sheet contents. However, insulin that is incorporated in polyelectrolyte multilayers rather forms amorphous aggregates as can be inferred from confocal fluorescence images. Only when insulin is the top-layer, formation of a fibrillar network can be observed after adding seeds to the buffer solution.

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The Transcription Factor Ultrabithorax Forms Extensible, Hierarchically Ordered Assemblies that are Readily Functionalized by Gene Fusion

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Proteins that naturally self-assemble to form materials are often challenging to produce as recombinant proteins, and require harsh conditions to instigate assembly in vitro, limiting their ability to be engineered for specific applications. Surprisingly, the Drosophila melanogaster Hox protein Ultrabithorax (Ubx) readily self-assembles in vitro in mild buffers, even though this protein does not form large aggregates as part of its native function. Ubx hierarchically associates to form materials that are ordered on the nanoscale to macroscale. The mechanical properties of Ubx fibers are tunable, and fibers can be constructed with a similar breaking stress and strain as natural elastin. Because Ubx self-assembles within hours in a mild buffer, other proteins can be incorporated into Ubx materials via gene fusion without compromising the ability of Ubx to assemble, or the functionality of the appended protein. A variety of proteins have successfully been incorporated into Ubx materials, ranging in structure, size, and charge. These functions can be easily patterned within the materials. This strategy has been used to control the behavior of cells in culture.

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An Optical Device Driven by Motor Protein

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Here, we developed a motor-protein-driven optical device. To design the device, we focused on body color change of fish. This phenomenon is caused by melanophore cells, which contains thousands of pigment granules. In a melanophore, pigments are transported by motor proteins along radially arranged microtubules. Thus, the distribution of pigments in a melanophore (i.e. color of a melanophore) is changed. By mimicking this molecular system, we tried to make an artificial melanophore from minimal components: motor proteins, microtubules and pigment granules. Nuclei of microtubule assembly were attached to micro-fabricated dot patterns, and microtubules were grown from them, which produced a radial array of numerous microtubules (Figure A). When stained micro-particles with flagellar dynein were introduced, those particles were transported along radially arranged microtubules and were gathered by ATP addition, which resulted in changing color patterns of each artificial melanophore (Figure B, C). Furthermore, we succeeded in drawing desired pictures on ''bio-display'', a display that one pixel is equivalent to one artificial melanophore, by adding ATP to particular pixels with caged ATP. This study has shown that a novel optical device can be constructed by arranging microtubules and motor protein molecules.